Glossitis of Military Working Dogs in South Vietnam: Histopathologic Observations

Frank A. Voelker, DVM, MS; Michael A. Stedham, DVM, MS; Farrel R. Robinson, DVM, PhD; Harold W. Casey, DVM, PhD

SUMMARY

Glossitis, known clinically as "redtongue," was studied in tissues from 34 military working dogs (MWD) in the Republic of Vietnam. This condition was manifested grossly by loss of lingual papillae on the dorsal margins of the rostral third of the tongue. Microscopically, the principal lesions consisted of loss of filiform papillae, hemorrhage and edema in the lamina propria, acanthosis, and cellular infiltration. The cause of glossitis remains unknown at this time.

Glossitis, or redtongue, as it is popularly termed, was a prevalent disease in MWD maintained in the Republic of Vietnam during the late 1960's and early 1970's. Clinically, the disease is manifested by redness of lingual epithelium, by disappearance of filiform papillae on the dorsal margins of the rostral third of the tongue, and sometimes, by ulceration.[16,18]

Naturally occurring disease of the tongue is not common in the dog and apparently has not been histologically characterized in the literature. Experimentally induced "blacktongue," an acute necrotizing glossitis, was produced by workers as early as 1917 by feeding rations deficient in B vitamins.[9,11,19] Afonsky[1,7] in 1955 refined the earlier efforts by experimentally producing glossitis in dogs fed rations deficient in niacin, folic acid, and pantothenic acid. The glossitis in these dogs was characterized by disappearance or atrophy of lingual papillae. In man, atrophic lingual changes are usually associated with systemic causes such as pellagra, sprue, pernicious anemia, anemias associated with parasitic infestations, and iron-deficiency anemias.[8,13]

The purpose in the present report is to describe the histologic observations in necropsy specimens from MWD submitted to the Armed Forces Institute of Pathology and in biopsy specimens obtained from tongues of MWD in Vietnam.

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From the Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington, DC 20306. Capt Voelker's current address is Experimental Pathology Department, Naval Medical Research Institute, Bethesda, MD 20014. Capt Voelker, Lt Col Robinson, and Lt Col Casey are in the US Air Force Veterinary Corps; LTC Stedham is in the US Army Veterinary Corps.

Address reprint requests to Armed Forces Institute of Pathology.

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Materials and Methods

Tissues from necropsies of 28 male and 2 female 2- to 7-year-old German Shepherd MWD were selected for detailed study after the diagnosis of glossitis was histologically confirmed. The dogs selected were considered representative of the population of both affected and unaffected MWD in Vietnam at that time with regard to sex, breed, and age. The reasons for death of the 30 dogs are given (Table 1).

TABLE 1—Causes of Death Among 30 Military Working Dogs Affected with Redtongue at Time of Necropsy

Cause	No. of dogs
Euthanasia for nonmedical reasons	14
Euthanasia for medical reasons	
Redtongue	9
Canine ehrlichiosis	4
Death from other causes	
Uremia	1
Strangulation (anoxia)	1
Septicemia, possibly secondary to canine ehrlichiosis	1
Total	30

Formalin-fixed specimens of tongue were studied under a dissecting microscope to correlate clinical history with gross and microscopic appearances. Representative specimens of each tongue were embedded in paraffin and sectioned 6 μm thick. Sections were stained with hematoxylin and eosin (H&E), Giemsa's, periodic acid-Schiff (PAS) with and without diastase, MacCallum-Goodpasture, Warthin-Starry, and Gomori's methenamine-silver stains. In addition, Pinkerton's stain for intracytoplasmic inclusions of *Ehrlichia canis* was utilized in some instances.[17]

Biopsy specimens of tongues were obtained from 2 dogs with glossitis of 2 days' duration and from 2 dogs with glossitis of unknown duration, but less than 1 year. These were processed in a manner similar to the necropsy specimens.

Results

Gross Observations—Formalin-fixed specimens had lesions similar to those described in clinical cases of the disease.[16,18] Focal regions of the margins of the rostral third of the tongue had shortened or missing papillae (Fig 1). In more severe cases, the focal regions coalesced so that the entire margin of the rostral third of the tongue was involved, and sometimes the central portion as well. Both fungiform and filiform papillae were affected. Normal filiform papillae have keratinaceous projections, the most apical and prominent

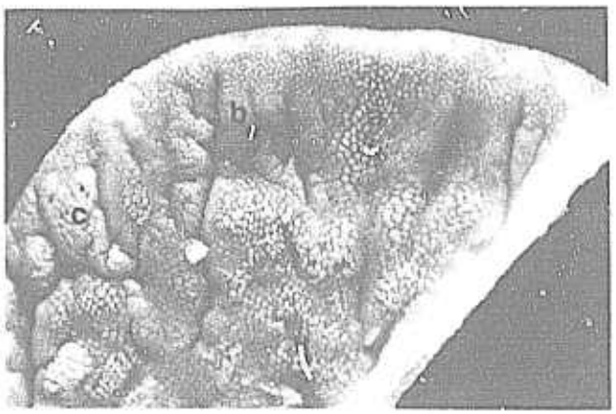

Fig 1—Subgross photograph of a formalin-fixed tongue from dog with redtongue. In some areas, filiform papillae are normal (a) in appearance, but in other areas there is loss of papillae (b). Area with severe acanthosis (c)

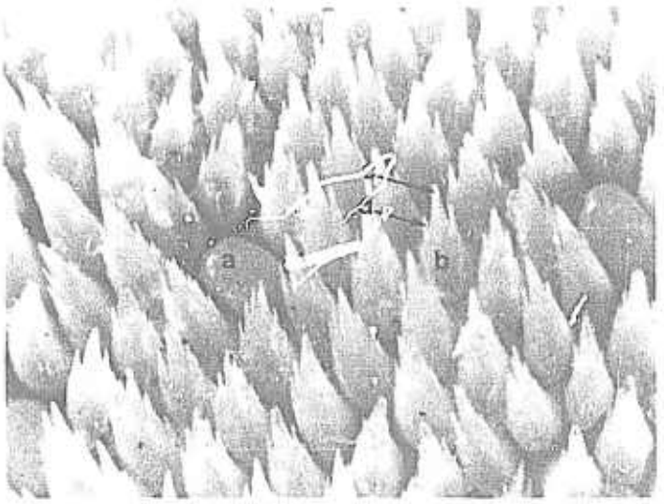

Fig 2—Subgross photograph of formalin-fixed tongue from a normal dog, illustrating fungiform papillae (a) and filiform papillae (b). On each filiform papilla are a primary papilla (c) and secondary papillae (d).

of which are primary papillae. Smaller secondary papillae are located adjacent to primary papillae (Fig 2). Closer examination of diseased specimens with a dissecting microscope revealed that the primary and the secondary papillae of each filiform papilla were missing in many instances where each papilla had not completely atrophied. This observation was especially characteristic of filiform papillae at the edges of the lesion.

Occasionally, in severely affected tongues, the regions of epithelium lacking in papillae presented a cauliflower or rugose proliferative appearance. Histologic examination later proved these areas to have very thickened acanthotic epithelium.

Microscopic Observations of Necropsy Specimens—Microscopically, the lesions could be divided into acute and chronic types. The acute lesions consisted of hemorrhage and edema of the lamina propria and vesiculation and ballooning degeneration of epithelial cells. Chronic lesions were characterized by loss of filiform papillae, acanthosis, and infiltration of the submucosa by inflammatory cells. The microscopic appearance of a normal canine tongue is illustrated (Fig 3).

Hemorrhage into the lamina propria of the filiform papillae (Fig 4) was seen in the tongues of 18 dogs. Although hemorrhage most often existed in regions of papillary atrophy, it also occurred in adjacent regions with normal papillae. In areas of hemorrhage, endothelial cells had pyknotic nuclei, and their cytoplasm was reduced and stained with eosinophilia. Other capillaries were dilated, and their endothelial cells were enlarged and had prominent hyperchromatic nuclei and abundant basophilic cytoplasm.

Intense edema of the connective tissue of the lamina propria (Fig 5) accompanied the capillary changes and imparted a rarefied and homogeneous appearance. The edema was sometimes of such severity that the epithelium separated from underlying connective tissue. A few neutrophils were frequently observed in the connective tissue surrounding the capillaries.

Vesiculation of the stratum corneum and underlying hydropic degeneration and vacuolization of noncornified epithelial cells (Fig 5) were seen in 7 of the

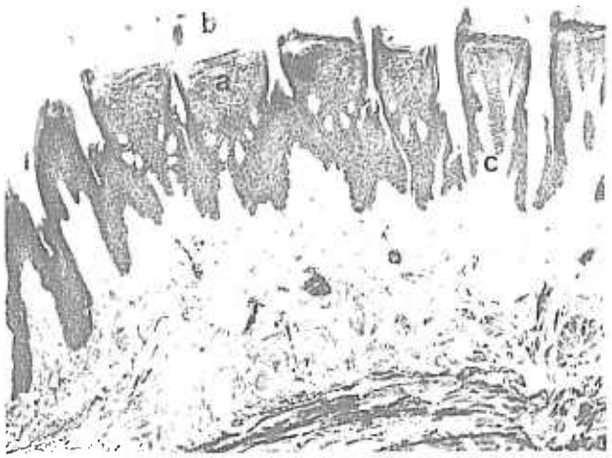

Fig 3—Section of normal canine tongue, illustrating filiform papillae (a), secondary papillae (b), which appear as superficial projections composed of keratin, and lamina propria (c), which is composed of finger like projections of connective tissue extending into the epithelium of the papillae. H&E stain; × 35.

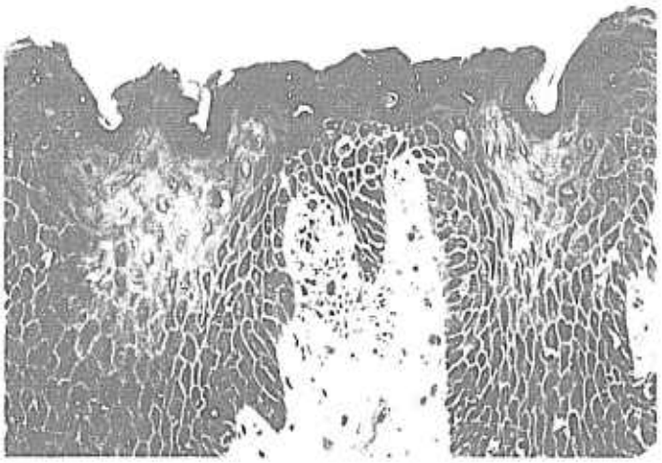

Fig 4—Hemorrhage into the lamina propria of an atrophic filiform papilla in tongue from dog with glossitis. The epithelium is moderately acanthotic. H&E stain; original magnification × 195.

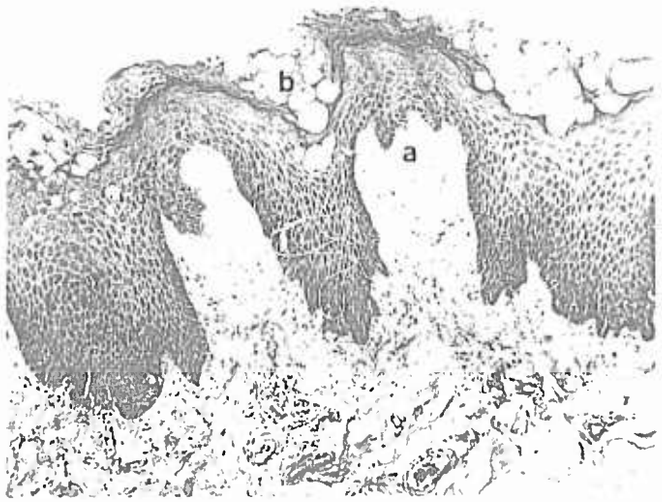

Fig 5—Lingual epithelium with severe edema in the lamina propria of atrophic filiform papillae (a) in tongue from dog with glossitis. Extensive vesiculation and ballooning degeneration are seen in the superficial epithelium (b). H&E stain; · 70.

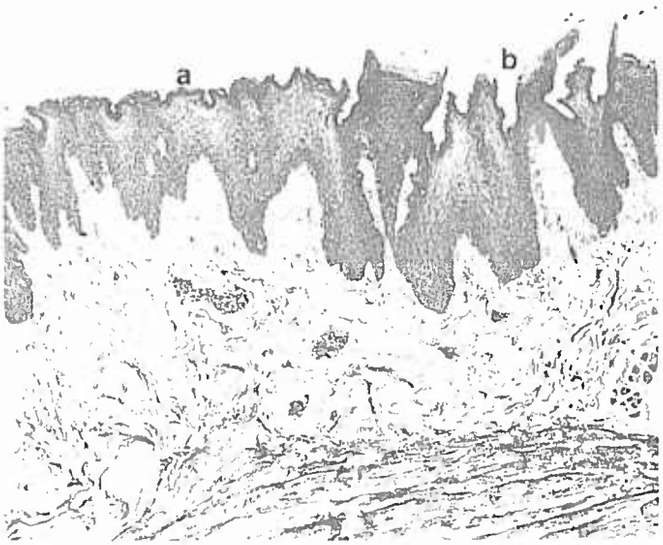

Fig 6—Section of tongue from dog with glossitis, illustrating focal atrophy of filiform papillae (a). Adjacent papillae are misshapen (b). H&E stain; · 35.

tongues. Regions of vesiculation and vacuolization were spaced in a regular manner over the epithelial surface and corresponded to interpapillary regions if filiform papillae had been present. The vesicular spaces were filled with a homogeneous, eosinophilic, proteinaceous material. Vesiculation usually occurred in tongues with the most severe edema of the lamina propria of filiform papillae.

Since loss of filiform papillae was one of the prime clinical characteristics of redtongue, it was consistently seen as a chronic lesion on microscopic examination. In mild cases, there was only focal loss of groups of papillae (Fig 6), but in more severe cases, loss of papillae was seen over all the epithelium at the plane of section. Although areas of atrophy were usually sharply demarcated, they were sometimes surrounded by a narrow zone of partially atrophied papillae. Fungiform papillae seemed to atrophy as quickly as the filiform papillae. The most mildly affected individual papillae exhibited only disappearance of secondary and tertiary papillae. More severely affected papillae were distorted and misshapen. The normally smooth surface contours of the stratum corneum became jagged and rough in appearance.

Acanthosis was a prominent microscopic component of redtongue and was more severe in the more extensively involved tongues. In mild cases, in which filiform papillae remained, the lingual epithelium between the lamina propria of individual papillae was acanthotic. Such downward proliferations of interpapillary epithelium into the submucosa persisted in severe cases, thus emphasizing the former locations of papillae before atrophy occurred. In some cases, it was so extensive it was pseudoepitheliomatous in nature. The stratum granulosum in the acanthotic epithelium was often absent or contained few granules of keratohyalin. The stratum corneum was parakeratotic and stained with more eosinophilia than normal. Randomly scattered epithelial cells were necrotic and were easily distinguishable by their shrunken, hyalin-like cytoplasm, which stained intensely with Giemsa's stain. Intracellular PAS-positive material (Fig 7) that was sensitive to diastase digestion and probably represented glycogen was seen in epithelial cells in the middle portion of the epithelium. This material was not consistently seen in all diseased specimens, but was frequently seen in specimens with hemorrhage in the lamina propria.

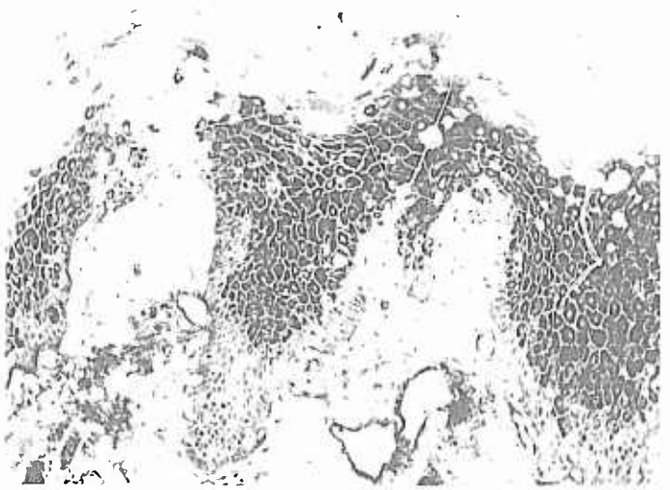

Fig 7—Numerous dark-staining glycogen-filled epithelial cells in the middle third of the lingual epithelium of tongue of dog with redtongue. Atrophy of papillae, acanthosis of the epithelium, and edema of the lamina propria are also shown. PAS stain; · 115.

In approximately 50% of the dogs, inflammatory cells were in the submucosa and consisted predominantly of plasma cells, lymphocytes, and monocytes (Fig 8). Macrophages and neutrophils were also seen, but in smaller numbers. Small focal infiltrations of lymphocytes also occurred between the epithelial cells in the basilar region of the epidermis. In these foci, there was epithelial cell necrosis. The basement mem-

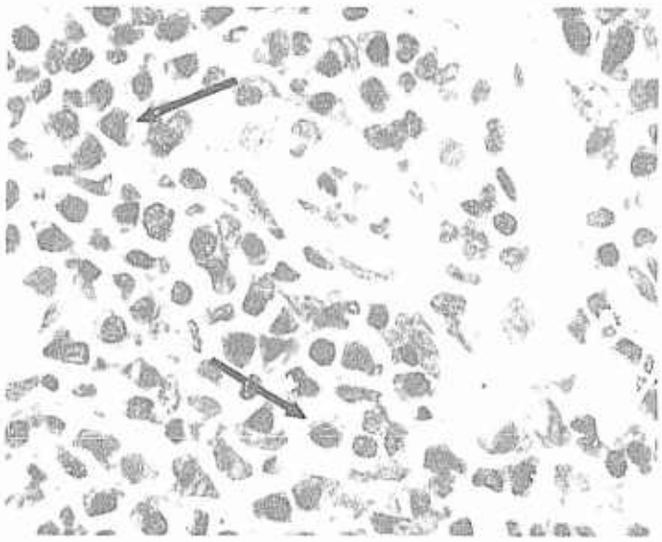

Fig 8—Submucosal cellular infiltrate composed predominantly of plasma cells (arrows) adjacent to a small blood vessel. H&E stain, × 880.

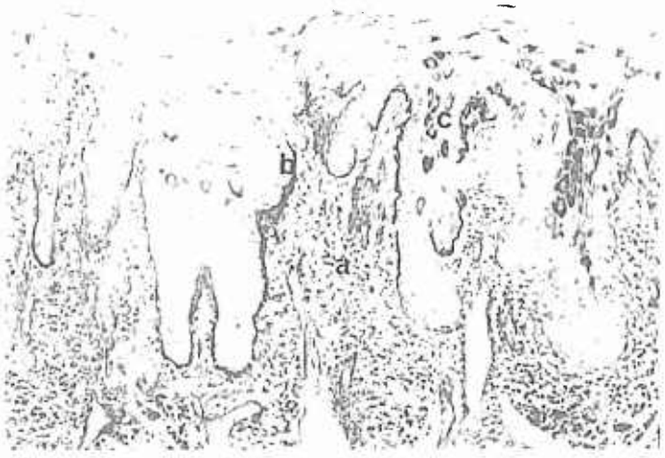

Fig 9—Severe chronic glossitis, with a uniform, diffuse infiltration of abundant inflammatory cells in the submucosal connective tissue (). Notice the thickened periodic acid-Schiff (PAS)-positive epithelial basement membrane (b). A few PAS-positive cells are in the acanthotic epithelium (c). PAS stain; × 115.

brane adjacent to these regions seemed to lose its smooth contour and to become jagged and even interrupted (Fig 9). The latter reaction was seen on both the dorsum and the ventrum of the tongues. Occasionally, tongues with more extensive inflammatory cell infiltrates contained individual muscle fibers in the submucosa that became necrotic and were shrunken and more eosinophilic in appearance.

Special stains did not reveal the presence of bacteria or other microorganisms in areas of inflammation or necrosis. Occasionally, saprophytic bacteria were observed embedded in the keratinized surface of lingual papillae, but their presence was considered to be normal.

Ulceration of the lingual epithelium, observed in 8 dogs, was associated with the presence of inflammatory cells in the submucosa. Basilar layers of lingual epithelial cells in these dogs were diffusely infiltrated by lymphocytes and neutrophils. Epithelium adjacent to the area of ulceration appeared thinned. Areas of ulceration were covered by a layer of fibrin intermixed with neutrophils.

Microscopic Observations of Biopsy Specimens—Although microscopic examination of the 4 biopsy specimens revealed many changes similar to those seen in necropsy specimens, some differences were apparent. In general, acanthosis was less severe and ulceration was more prominent. The inflammatory cell infiltrate was minimal, consisting predominantly of neutrophils adjacent to areas of ulceration.

Discussion

The cause of redtongue remains open to speculation. The absence of viral inclusions in cells or of primary bacterial invasion in tissues histopathologically examined does not rule out the possibility of a microorganism as a causative agent. In this respect, canine ehrlichiosis caused by *E canis* had been a problem of importance in military dogs in Vietnam.[11,20] Efforts made to correlate the presence of ehrlichiosis with glossitis were not successful. Although some of the dogs with redtongue also had canine ehrlichiosis, the typical morula-like inclusions in monocytes, neutrophils, and endothelial cells were not demonstrated in sections of tongue by the use of Pinkerton's stain. Complete histopathologic examinations were performed on all dogs. Lesions were not consistently seen in other organs of dogs with redtongue that were not seen in nonaffected MWD routinely examined from Vietnam.

Vesiculation in the epithelium and hemorrhage and edema in the lamina propria of filiform papillae are changes indicative of a toxic irritant. Exposure to a toxic agent was considered as a possible cause, but examination of clinical histories of the dogs in the present study and conversations with veterinarians directly observing the dogs did not reveal consistent common factors.

Since removing dogs from direct sunlight and keeping them confined to the shade seemed to alleviate the condition, efforts were made to compare microscopic lesions of redtongue with those of light-related skin disease. Early responses of skin with sunburn and phototoxic reactions in man include degeneration of scattered epithelial cells, intraepidermal vesiculation, vasodilation, edema, and accumulation of glycogen in epidermal cells.[19] Such lesions were observed in the tongues of dogs with redtongue. The plasma and lymphoid cell infiltrates in the submucosa of affected tongues were somewhat similar to those described for photoallergic reactions in the skin of man[12] and, in our experience, to those of chronic canine solar dermatitis (collie nose). Recently, lesions which closely resemble those of glossitis have been experimentally produced in dogs by means of short- and long-term exposure to artificial sunlight.[15]

Although redtongue has clinical and histologic similarities to nutritional human atrophic glossitis[8] and to experimentally induced canine vitamin B deficiency,[1-7] one should be cautious in assuming that it has a similar cause and pathogenesis. In studies by Afonsky,[1-7] dogs

with riboflavin deficiency had lesions most similar to redtongue. He noted small, patchy, discrete atrophic areas on the rostral two-thirds of the dorsum of the tongue involving both the fungiform and the filiform papillae. Microscopically, rarefaction of adjoining mucosa was also reported. The atrophic changes were more diffuse in dogs with niacin, folic acid, and pantothenic acid deficiency. Hemorrhage into the lamina propria of filiform papillae was not described in dogs with any of the vitamin deficiencies, and the submucosal inflammation was apparently not as prominent as in dogs with redtongue.

The MWD are fed a ration formulated and tested for nutritional adequacy. It is conceivable that the prolonged, hot storage conditions that prevailed in Vietnam could have led to degradation of unstable vitamin components in the dog food. Although many of the dogs had either hookworm or whipworm infestations, most were not anemic, as revealed by hematologic analyses before dogs died. Lack of anemia seems to diminish the probability of iron or riboflavin deficiencies.[13] The occurrence of the condition in several dogs within a week of arrival in Vietnam and also the reported apparent unresponsiveness to therapy of dogs with redtongue to vitamin B therapy also diminish the possibility of nutritional deficiency as a cause of the disease.[18]

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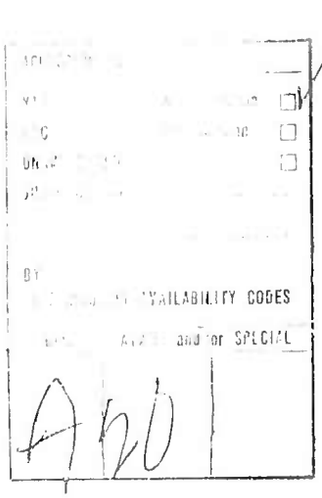
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